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Iranian Scorpion (*Odontobuthus bidentatus*) crude venom change the redox potential of MCF-7 breast cancer cell line and induce apoptosis

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ABSTRACT

New natural substances obtained from scorpion venoms could be promising approaches for the treatment of cancers. Scorpion venom is a fully mixed compound that containing enzymes, non-enzymes, ions, and other organic compounds that induces apoptosis and necrosis in mammalian cells. In this study, the cytotoxicity effects, redox potential, and the ability of apoptosis induction of *Odontobuthus bidentatus* scorpion venom on MCF-7 cells were investigated. To do this, the MCF-7 cells were treated with the scorpion venom. MTT and neutral red assays was used to evaluate the cytotoxicity. Catalase, GSH and NO assays are used to determine the cells redox potential. Caspase-3 and cytochrome c release assays were exploited to investigate the apoptosis. The results of MTT and neutral red tests showed that O. bidentatus crude venom has cytotoxic effects on MCF-7 cells. Moreover, the results of catalase, GSH and NO assays showed that the crude venom could change the redox potential of MCF-7 cells, dose dependently which eventually lead to apoptosis. Also, the results of caspase-3 and the release of cytochrome c confirmed cell apoptosis. These results suggest that O. bidentatus venom is a suitable source of apoptosis-inducing compounds.

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Abbreviations

LAAO, l-amino acid oxidase; NO, Nitrite oxide; GSH assay, Reduced glutathione assay; GPx, Glutathione peroxidase; GSSG, Oxidized glutathione;

Introduction

Cancer is a major public health problem caused mortality in developed countries (1). Currently, in Iran, more than 110,000 cancer cases occurred and more than 56,000 patients died (2). In 2020, there were 2.3 million women diagnosed with breast cancer and 685000 deaths globally (3). Conventional antitumor therapies such as chemotherapy, radiotherapy and surgery are used to treat cancer but these methods have some disadvantages (4). Recently researchers focused on natural components as a promising new approach for the treatment of cancers (5). For example, some natural compounds such as 3,3′-Diindolylmethane (DIM) derived from cruciferous vegetables such as broccoli (6), Biochanin A extracted from red clover (Trifolium pratense) (7), l-amino acid oxidase (LAAO) derived from moccasin snake venom (8) have anti-cancer properties.

Scorpions and their venoms have been used in traditional medicine for thousands of years in China, India and Africa (9). Their venom contains a complex mixture of peptides, enzymes, mucoproteins, and some other proteins (9) which are biologically active and have anticancer properties (10). An increasing number of studies have shown that scorpion venoms and toxins can decrease cancer growth, induce apoptosis and inhibit cancer progression and metastasis in vitro and in vivo (9, 10). Anticancer peptides derived from scorpion venom are important resources of natural components for the design of tumor-targeting drugs (11). For example, the BmTx3, from *M. martensii*, AmmTx3 from *Androctonus mauritanicus*, and Bekm-1 from *Mesobuthus eupeus* are some peptides that inhibit the growth of cancer cells and induce apoptosis (12, 13). Chlorotoxin (CTX or CLtx) obtained from *L. quiquestraiatus hebraeus* venom is another protein derived from scorpion venom that has anti-cancer properties (14).

Scorpion venom creates a stressful environment in cancer cells which triggers the generation of free radicals and hence reactive oxygen species (15). Changing in the redox potential of treated cells is the leading cause of cell death and could be a potential therapeutic approach for cancer therapy (16). In addition, it is important to note that changing in the redox potential induces DNA damage and change the drug sensitivity of many cancer types (17).

Scorpions are primitive arthropods belonging to order scorpions and class Arachnida. More than 1500 different species of scorpions are known which belong to 16 families (9).

Odontobuthus bidentatus is a member of the *Odontobuthus* genus and the *Botheida* family which is found in Iran (Khuzestan, Kerman, Bushehr, Fars, Ilam, and Hormozgan provinces) and Iraq (Baghdad province) (18).

In the present study, the effects of the venom of *O.bidentatus* scorpion on the MCF-7 cell redox potential and apoptosis induction were investigated.

Materials and Methods

Preparation of Scorpion Venom

O. bidentatus scorpions were collected from Khuzestan and Hormozgan provinces (Iran) after obtaining permission from the Ministry of Health, Govt. of Iran. This study was registered in Iranian Research Institute for Information Science and Technology (https://irandoc.ac.ir/) with identifier 1367381. Scorpion venom was milked using electrical stimulation from telsons. The venom was centrifuged at 8000×g for 15 min at 4 °C. Then, the supernatant was lyophilized and stored at –20°C. In order to prepare a toxic solution, the lyophilized powder was dissolved in a serum- and phenol-free medium and the protein concentration was determined using the Bradford method (19).

Cell Culture

The MCF-7 cell line was purchased from the Cell Bank of the Iranian Biological resource center, Tehran, Iran. Cells were cultured in T25 plastic flasks containing DMEM-F12, supplemented with 10% heat-inactivated fetal bovine serum, and 10µg/ml penicillin-streptomycin. The cells were incubated at 37 °C with 5% CO^2 and 80% humidity. The culture medium was replaced three times a week. Cell counting was performed using a hemocytometer (HGB, Germany).

Cell Treatment

Cell treatment was performed in sterile plastic plates (SPL, Korea). For each test, an appropriate number of cells was seeded and incubated for a night at 37°C, 5% CO_2, and 80% humidity. After incubation, the medium was replaced with the fresh serum-free medium containing different concentrations of the venom.

MTT Reduction Assay

MTT assay (20) was used to determine the cytotoxic effects of *O. bidentatus* venom. Briefly, 3×10^4 cells were seeded in a 96-well plate and incubated overnight at 37°C, 5% CO_2, and 80% humidity.

Then, the old culture media were exchanged with a serum-free media containing different final concentrations of venom (10, 20, 50 and 100 µg/ml). In the next step, 10µl of MTT stock solution (5mg/ml) was added to each well and incubated at 37°C for 2 hours. After that, 200µl DMSO was added to each well and placed in a shaker incubator for 2 hours under dark conditions. Then, absorption was measured at 595nm by a microplate reader (Biotech, USA).

Neutral Red Uptake Assay

Repetto et al. was described the neutral red uptake assay (21). First, 3×10^4 cells were seeded in a 96-well plate for a night at 37 °C. Then, the old media were removed and fresh serum-free media containing different final concentrations of the venom (10, 20, 50 and 100 µg/ml) were added to each well. Thereafter, 5µl of neutral red solution (5mg/ml) was added to each well. After 1h of incubation at 37 °C and formation of red crystals, the culture medium was carefully removed. Then, 100 µl of stabilizing solution (37% formaldehyde and 10% $CaCl_2$ in ddH_2O) was added to each well and removed after 1 min. To dissolve the red crystals, 100µl of solvent buffer (5% acetic acid) was added to each well and the absorbance was measured at 540nm by a microplate reader (BioRad, USA).

Nitrite Oxide (NO) Assay

Ding et al. method (22) was used to measure the amount of nitrite oxide released to the culture media. To achieve this aim, 3×10^4 cells were seeded in a 96-well plate and incubated overnight at 37 °C. Then, the media were discarded and fresh media containing different concentrations of the venom (10, 20, 50 and 100 µg/ml) were added. After 24h, the culture medium was removed and transferred to 1.5 ml microtubes. After centrifugation at 500×g for 5 min at 4 °C, 100 µl of the supernatant was transferred to a 96-well plate and mixed with an equal volume of the Griess reagent solution (0.04g/ml Griess reagent in PBS, pH 7.4, Sigma Aldrich). The plate was incubated for 10 min at room temperature. Absorption was measured at 540nm by a microplate reader (Bio-Rad, USA). Nitrite oxide concentration was measured in untreated and treated cells using the standard nitrite oxide reference curve and expressed in µM/ml.

Reduced Glutathione (GSH) Assay

GSH level was measured according to the Sedlak and Linsay method (23). In this regard, 5×10^5 cells were seeded in a 24-well plate. After a night incubation, the old media were replaced with fresh media containing different concentrations of the venom (10, 20, 50 and 100µg/ml). The plate was incubated at 37°C for 24 h. Then, the cells were collected, washed twice with PBS (pH 7.4), and centrifuged at 500×g for 5 min at 4°C. The collected cells were incubated for 20 min at -20°C and lysed using 200µl of chilled cell lysis buffer (20mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM EDTA and 1% Triton X-100) and incubated for 30 min at room temperature. The cells were then sonicated for 10–15 min and centrifuged at 2000×g for 10 min and the supernatant was collected. Protein concentration was calculated using the Bradford method. Then, an equal volume of TCA (Trichloroacetic acid) 10% was added to the supernatant and incubated at 4 °C for 2 h. The contents were collected by centrifugation at 500×g for 15min. Then, 20µl of the sample was mixed with 75µl lysis buffer, 55µl Tris buffer (pH 8.5) containing 0.02 M EDTA and 25µl DTNB [5,5'-dithiobis-(2-nitrobenzoic acid)]. The absorption was measured at 412nm by a microplate reader (BioRad, USA). The result was expressed in µg GSH/mg protein using molar extinction coefficient of 13,600.

Catalase Activity Assay

Catalase enzyme activity was measured using Sinha method (24). Like GSH assay protocol, 5×10^5 cells were seeded and treated with different concentration of crude venom. The cell lysate was prepared as described in GSH assay protocol and the protein content was determined using Bradford method. Then, 5µl of the sample was mixed with 50µl of lysis buffer, 20µl of distilled water and 25µl of 15% hydrogen peroxide. The samples were shaken thoroughly and incubated at 37°C for 2 min. Then, 100µl of dichromate reagent (0.1M potassium dichromate in glacial acetic acid) was added to the samples and the mixture was placed in a 100°C water bath for 10–15 min until the color of the samples changed to pale green. Then, 100µL of the samples were transferred to a 96-well plate and absorbance was read at 570 nm by a microplate reader (BioRad, USA). Catalase enzyme activity was expressed in µM of consumed H_2O_2 per min per mg of protein (Molar extinction coefficient was 43.6).

Determination of Caspase-3 Activity

Caspase-3/CPP32 kit (Biovison, USA) was used to determine the activity of the caspase-3 enzyme. To achieve this aim, 1×10^6 cells were seeded in a 6-well plate and incubated for a night at 37 °C. Thereafter, the media were carefully removed and fresh serum- and phenol-free medium with various concentrations of the venom (10, 20, 50 and 100µg/ml) were added to the wells and incubated at 37 °C for 24 h. The cells were then collected and transferred to 1.5ml microtubes and centrifuged at 500×g for 5 min at 4 °C and washed twice with PBS (pH 7.4). Then, the caspase-3 enzyme activity was determined according to the kit manufacturer's manual. Ultimately, the absorbance of samples was measured at 400nm by a microplate reader (BioRad, USA).

Estimation of Cytochrome c Released from Mitochondria

The Biovison's cytochrome c releasing apoptosis assay kit (Biovison, USA) was used to estimate the amount of cytochrome c released from mitochondria to the cytosol. Like caspase-3 activity assay, 1×10^6 cells were seeded and treated with different concentration of crude venom. Then, 1ml of 1X cytosol extraction buffer containing DTT and protease inhibitor (provided by the kit) was added to the cells and incubated for 10 min on ice. The cells were then homogenized in a Dounce tissue grinder in cold conditions. The homogenized cells were then transferred to 1.5ml micro-tubes and centrifuged at 700×g for 10 min at 4°C and the supernatant was transferred to the new micro-tubes. The samples were centrifuged at 10,000×g for 30 min at 4°C and the supernatant was collected as a cytosolic fraction.

The protein concentration of each sample was determined using the Bradford method. Then, using indirect ELISA as well as using monoclonal antibodies against cytochrome c (provided by the kit), the amount of released cytochrome c to cytosol in venom treated cells was compared with the untreated cells.

Statistical Analysis

All experiments have been conducted in triplicate and reported as Mean ± SD. All data were analyzed using Graphpad Prism 8 Software (La Jolla, CA, USA). MCF-7 cells were treated with different concentrations of the venom and the results were compared with the control group using one-way ANOVA and Tukey tests. $P<0.05$ was determined as significant.

Results

MTT Reduction Assay

The results of MTT assay results showed that the venom of *O.bidentatus* reduces the viability of the MCF-7 cell line in a dose dependant manner (Fig. 1-A). As shown in Fig. 1-A, the survival rate of MCF-7 cells after exposure to 10, 20, 50 and 100µg/ml of the venom was 90.67, 77.00, 67.33 and 53.67 percent, respectively.

Neutral Red Uptake Assay

The results of the neutral red uptake assay showed that *O.bidentatus* scorpion venom inhibited the growth of MCF-7 cell line (Fig. 1-B). The inhibition percent of MCF-7 cells following treatment with 10-100µg/ml of the venom was 10.67, 23.64, 34.67, and 46.35 percent, respectively.

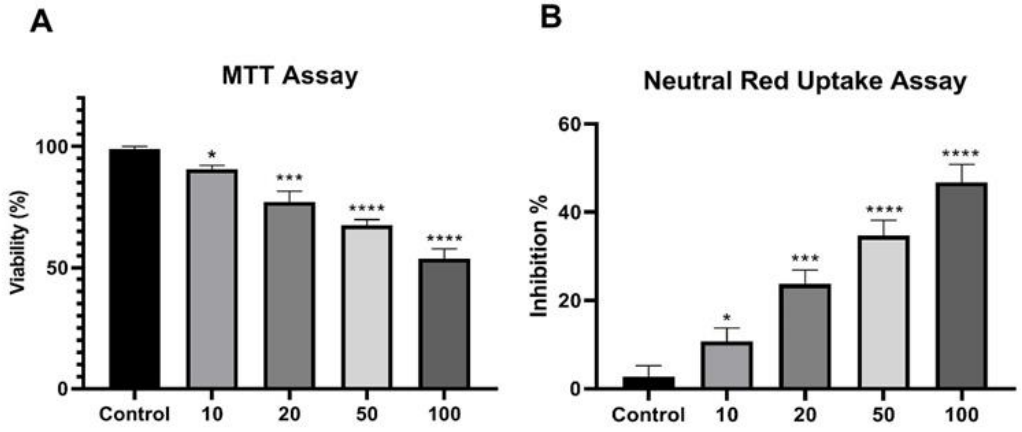

*Figure 1Figure 1. A) MCF-7 cells viability following treatment with 10-100µg/ml of O.bidentatus scorpion venom. The results showed that the viability of MCF-7 cells decreased significantly. B) Inhibitory effects of different concentrations of the of O.bidentatus crude venom on MCF-7 cells. The results showed that scorpion venom inhibited the growth of MCF-7 cell line dose dependently. * P<0.05, *** P<0.001, **** P<0.0001*

Nitrite Oxide (NO) Assay

The amount of NO released into the culture media was measured. The results showed that treatment of MCF-7 cells with *O.bidentatus* crude venom increased the concentration of nitrite oxide in media. As shown in Fig. 2-A, the concentrations of NO accumulated in the media were 7.33, 9.00, 24.33 and 35.12µg/ml following treatment of the cells with 10, 20, 50 and 100 µg/ml of the venom, respectively.

Catalase Activity Assay

The results of Fig. 2-B showed that the activity of catalase enzyme in MCF-7 cells decreased significantly after exposure to *O.bidentatus* venom (P<0.0001). Catalase activity of MCF-7 cells treated with 10, 20, 50 and 100µg/ml were 31.33, 29.67, 22.33 and 14.67 µM of consumed hydrogen peroxide/min/mg protein, respectively.

Reduced Glutathione (GSH) Assay

Treatment of MCF-7 cells with different concentration of the *O.bidentatus* crude venom decrease the concentration of the reduced glutathione in the dose dependant manner (Fig. 2-C). The results showed that the concentration of GSH in the venom treated groups was 0.42, 0.38, 0.27 and 0.18µg GSH/mg protein following the treatment of the cells with 10, 20, 50 and 100 µg/ml of the venom, respectively.

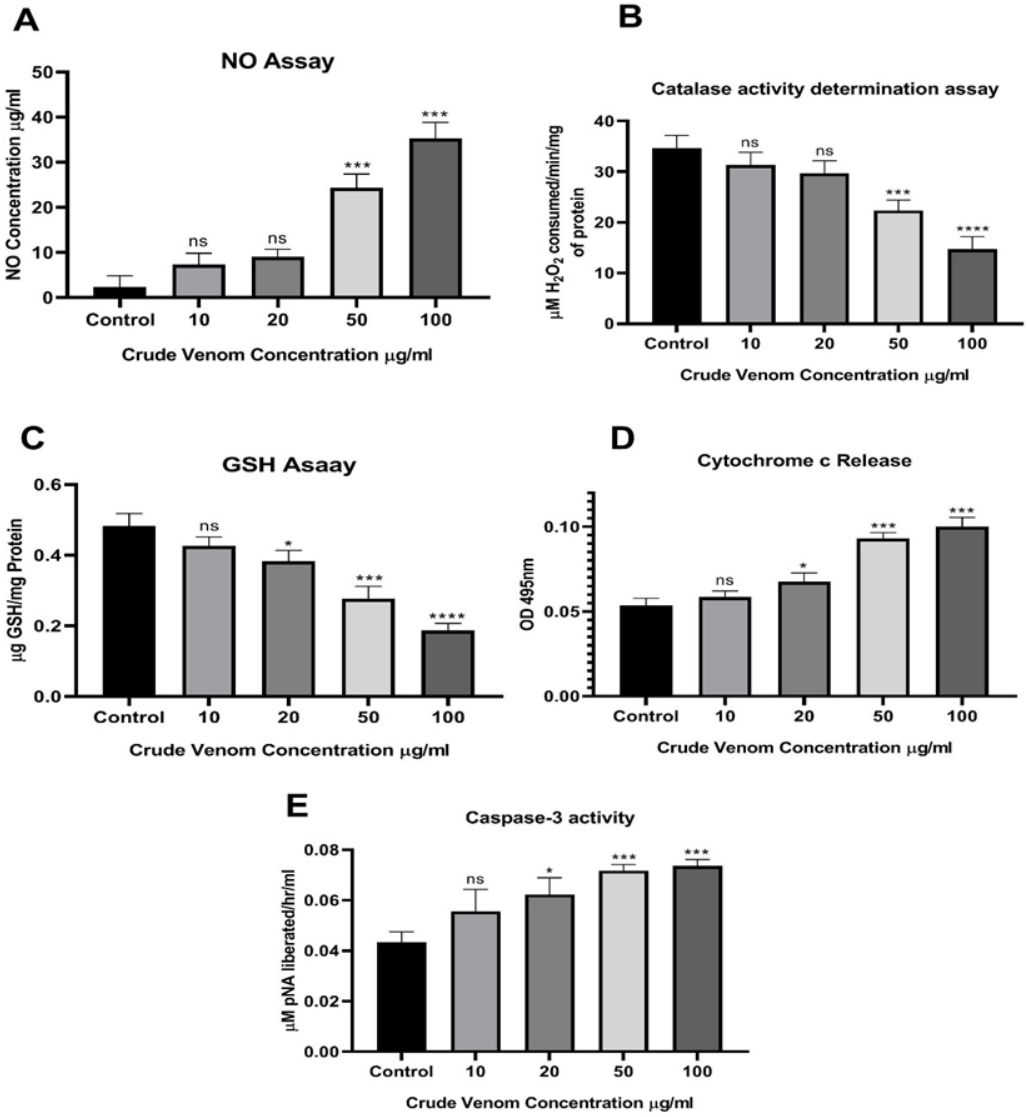

*Figure 2Figure 2. A) The concentration of nitrite oxide released by the MCF-7 cells following treatment with different concentrations of the O.bidentatus crude venom. B) Estimation of catalase activity in MCF-7 cells following treatment with different concentration of scorpion crude venom. C) GSH concentration in the venom treated groups was significantly decreased as the venom concentration increased. GSH concentration has been expressed as µg GSH/mg protein. D) Estimation of the amount of released cytochrome c from mitochondria into cytoplasm using indirect ELIZA. Treatment of MCF-7 cells with different concentrations of the venom led to the increased mitochondrial release of the cytochrome c. E) The results showed that caspase 3 activity increased in the venom-treated cells compared to untreated cells following treatment with the scorpion venom. ns: not significant, * P<0.05, *** P<0.001, **** P<0.0001*

Estimation of Cytochrome c Released from Mitochondria

As shown in Fig. 2-D, treatment of MCF-7 cells with the scorpion venom increased the amount of cytochrome c release into cytosol. The treatment of the MCF-7 cells with 20-100µg/ml of the venom increased the leakage of cytochrome c from mitochondria to cytosol in a dose-dependent manner.

Determination of Caspase-3 Activity

Treatment of MCF-7 cells with *O.bidentatus* crude venom significantly increased the activity of the caspase-3 enzyme (Fig. 2-E). The activity of the enzyme following the treatment with 10, 20, 50 and 100 µg/ml of the venom was 0.055, 0.062, 0.071, and 0.073µm of p-NA liberated/h/ml at 37 °C, respectively.

Discussion

Due to the presence of various types of bioactive molecules, such as proteins, small peptides, and amines, scorpion venom is a potent anticancer agent (25). These peptides and proteins in the scorpion venoms inhibit the growth of cancer cell lines via three different mechanisms: 1) blocking a specific ion channel (26), 2) binding to a specific site in the plasma membrane of cancer cells and thus inhibiting their invasion (27), and 3) activating mitochondrial apoptotic pathway (28).

The results of this study showed that the venom of *O. bidentatus* scorpion has a cytotoxic effect on breast cancer cells (MCF7). It was shown that the venom of this scorpion changes the redox potential and as a result induces apoptosis in the mentioned cell. Changes in the NO, GSH, and cytochrome c concentration and catalase and caspase-3 activity proved that apoptosis was induced in the MCF-7 cell line following treatment with scorpion venom. Also, the results confirmed that the apoptosis was induced through the mitochondrial pathway.

In this study, the results of MTT and neutral red uptake assays showed that the venom of *O. bidentatus* has cytotoxicity effect on MCF-7 cells in the dose dependant manner. The results of MTT assay was confirmed by the neutral red uptake assay. Results of MTT assay showed that the viability of MCF-7 cells decreased significantly following the treatment with the venom and the results of neutral red uptake assay, which is more sensitive than MTT (21), showed the same results.

We previously showed that the venom of *O.bidentatus* has a cytotoxic effect on the HepG2 cells and induces apoptosis in these cells, dose-dependently (29). Salarian et al. showed that the venom of *Odontobuthus doriae* scorpion has a cytotoxic effect on HepG2 cells (30). Salehi et al. showed that the *O. bidentatus* scorpion crude venom had a significant cytotoxic effect on MCF-7, A549, and AGS cell lines (31). Zargan et al. indicated that *O. doriae* crude venom could reduce MCF7 cell viability in the dose dependant manner (32).

Treatment of MCF-7 cells with 50 and 100µg/ml of scorpion venom significantly increased the release of NO into culture medium ($P< 0.001$). NO as an oxidative agent increases the permeability of the mitochondrial membrane and thus could be the primary signs of apoptotic pathway in the treated cells (33). Zargan et al. showed that the venom of *O. doriae* increases the NO release from the human neuroblastoma cell line (SH-SY5Y) to the culture medium (34). Elevation of NO concentration in cancer cells is the leading cause of apoptosis and the results of this study showed that treatment of MCF-7 cells with scorpion venom increase the NO concentration in the media.

Catalase is a significant enzyme in the way of oxidative stress and is the most important scavengers of oxidative radicals. Resistance to the ROS signalling pathway through the elevated catalase activity is one of the characteristics of cancer cells, and thus reduction of the activity of this enzyme could be a promising approach for the inhibition of the growth of cancer cells (35). Our results showed that the activity of catalase enzyme in MCF-7 cells decreased significantly following exposure to *O.bidentatus* venom ($P<0.0001$). This finding was in accordance with other studies. For example; Abdel-Rahman et al. investigated the effects of the venom of *Maurus palmatus* scorpion and showed that the activity of catalase enzyme was reduced in the treated cells (36). Zargan et al. also showed that the venom of *O. doriae* scorpion reduced the activity of catalase enzyme in MCF-7 cell line (32).

Here, we found that the venom of O. bidenatatus scorpion decreases the reduced glutathione GSH in MCF-7 cells, dose dependently. It was shown that reduction of GSH in cancer cells could lead to apoptosis (37, 38). Moreover, it is demonstrated that scorpion venom can increase the activity of glutathione peroxidase (GPx), which is responsible for the conversion of GSH to the oxidized glutathione (GSSG) in cancer cells (39).

Amina et al. showed that subcutaneous administration of *Androctonus australis* hector scorpion venom decreases GSH in mice sera (40). Collectively, the results of NO, catalase, and GSH show that the redox potential of the MCF-7 cells has undergone the changes following the treatment of the cells with the venom of the *O. bidenatatus* scorpion. The changes in redox potential, in turn, drive the cells to apoptosis.

In this study, it was shown that by increasing the concentration of scorpion venom, the activity of the caspase-3 enzyme is significantly increased ($P<0.001$). Moreover, concentrations of 50 and 100µg/ml of *O. bidenatatus* crude venom significantly increased the release of cytochrome c from mitochondria to cytosol ($P<0.001$), which is consistent with the results of the NO release assay.

Studies have shown that scorpion venom contain multiple components that inhibit the growth of cancer cells and induce apoptosis and prevent metastatic cancer cells both in in vitro and in vivo conditions (25). Currently, some proteins and peptides isolated from scorpion venom are used to develop new anticancer drugs (25).

Conclusion

In this study, it was observed that *O. bidentatus* scorpion venom has cytotoxic effects and decrease the viability of cancer cells in the dose depandant manner. Here, we found that the *O. bidentatus* scorpion crude venom has the compounds that can change the redox potential of cancer cells and hence, induce apoptosis in breast cancer cells through the mitochondrial pathway; however, it demands more investigations to isolate the active components of the venom.

Declarations

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Conflicts of interest
Not applicable.

Authors' Contributions

H.K.A involved in conceptualization, methodology, and writing the original draft, J.Z and A.B involved in conceptualization and methodology, E.Z, M.M, and A.H involved in methodology.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34.
2. Zendehdel K. Cancer statistics in IR Iran in 2018. Basic & Clinical Cancer Research. 2019;11(1):1-4.
3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA: a cancer journal for clinicians. 2020;70(1):7-30.
4. Morrison R, Schleicher SM, Sun Y, Niermann KJ, Kim S, Spratt DE, et al. Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. Journal of oncology. 2011;2011.
5. Karikas G. Anticancer and chemopreventing natural products: some biochemical and therapeutic aspects. J BUON. 2010;15(4):627-38.
6. Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, van den Brandt PA. Epidemiological studies on brassica vegetables and cancer risk. Cancer Epidemiology and Prevention Biomarkers. 1996;5(9):733-48.
7. Heinonen S-M, Wähälä K, Adlercreutz H. Identification of urinary metabolites of the red clover isoflavones formononetin and biochanin A in human subjects. Journal of Agricultural and Food Chemistry. 2004;52(22):6802-9.
8. Li L, Huang J, Lin Y. Snake venoms in cancer therapy: past, present and future. Toxins. 2018;10(9):346.
9. Ding J, Chua P-J, Bay B-H, Gopalakrishnakone P. Scorpion venoms as a potential source of novel cancer therapeutic compounds. Experimental biology and medicine. 2014;239(4):387-93.
10. Gomes A, Bhattacharjee P, Mishra R, Biswas AK, Dasgupta SC, Giri B, et al. Anticancer potential of animal venoms and toxins. 2010.
11. Ortiz E, Gurrola GB, Schwartz EF, Possani LD. Scorpion venom components as potential candidates for drug development. Toxicon. 2015;93:125-35.
12. Liu Z, Tao J, Ye P, Ji Y. Mining the virgin land of neurotoxicology: a novel paradigm of neurotoxic peptides action on glycosylated voltage-gated sodium channels. Journal of Toxicology. 2012;2012.
13. Hmed B, Serria HT, Mounir ZK. Scorpion peptides: potential use for new drug development. Journal of toxicology. 2013;2013.
14. Cohen G, Burks SR, Frank JA. Chlorotoxin—A multimodal imaging platform for targeting glioma tumors. Toxins. 2018;10(12):496.
15. Liou G-Y, Storz P. Reactive oxygen species in cancer. Free radical research. 2010;44(5):479-96.
16. de Pinto MC, Tommasi F, De Gara L. Changes in the antioxidant systems as part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco Bright-Yellow 2 cells. Plant physiology. 2002;130(2):698-708.

17. Pelicano H, Carney D, Huang P. ROS stress in cancer cells and therapeutic implications. Drug resistance updates. 2004;7(2):97-110.
18. Lourenco WR, Pezier A. Taxonomic consideration of the genus Odontobuthus Vachon (Scorpiones, Buthidae), with description of a new species. Revue suisse de Zoologie. 2002;109(1):115-25.
19. Kruger NJ. The Bradford method for protein quantitation. The protein protocols handbook: Springer; 2009;17-24.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods. 1983;65(1-2):55-63.
21. Repetto G, Del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature protocols. 2008;3(7):1125-31.
22. Ding AH, Nathan CF, Stuehr D. Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production. The Journal of Immunology. 1988;141(7):2407-12.
23. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Analytical biochemistry. 1968;25:192-205.
24. Sinha AK. Colorimetric assay of catalase. Analytical biochemistry. 1972;47(2):389-94.
25. Al-Asmari A, Khan H, Manthiri R. Effect of Androctonus bicolor scorpion venom on serum electrolytes in rats: a 24-h time–course study. Human & experimental toxicology. 2016;35(3):293-6.
26. Jäger H, Dreker T, Buck A, Giehl K, Gress T, Grissmer S. Blockage of intermediate-conductance Ca2+-activated K+ channels inhibit human pancreatic cancer cell growth in vitro. Molecular pharmacology. 2004;65(3):630-8.
27. Deshane J, Garner CC, Sontheimer H. Chlorotoxin inhibits glioma cell invasion via matrix metalloproteinase-2. Journal of biological chemistry. 2003;278(6):4135-44.
28. Gupta SD, Gomes A, Debnath A, Saha A, Gomes A. Apoptosis induction in human leukemic cells by a novel protein Bengalin, isolated from Indian black scorpion venom: through mitochondrial pathway and inhibition of heat shock proteins. Chemico-biological interactions. 2010;183(2):293-303.
29. Alikhani HK, Bidmeshkipour A, Zargan J. Cytotoxic and apoptotic induction effects of the venom of Iranian scorpion (Odontobuthus bidentatus) in the Hepatocellular carcinoma cell line (HepG2). International Journal of Peptide Research Therapeutics. 2020:1-10.
30. Salarian AA, Jalali A, Mirakabadi AZ, Vatanpour H, Shirazi FH. Cytotoxic effects of two Iranian scorpions Odontobuthusdoriae and Bothutus saulcyi on five human cultured cell lines and fractions of toxic venom. Iranian journal of pharmaceutical research: IJPR. 2012;11(1):357.
31. Salehi-Najafabadi Z, Goudarzi HR, Sajadi M. Evaluation of in vivo Lethality and in vitro Cytotoxic Effect of Odontobuthus bidentatus Scorpion Venom. Archives of Razi Institute. 2022;77(1):19-26.
32. Zargan J, Umar S, Sajad M, Naime M, Ali S, Khan HA. Scorpion venom (Odontobuthus doriae) induces apoptosis by depolarization of mitochondria and reduces S-phase population in human breast cancer cells (MCF-7). Toxicology in Vitro. 2011;25(8):1748-56.
33. Jiang X, Zheng D, Lin J. Effects of nitric oxide on mitochondrial permeability transition and cytochrome C of human hepatocellular carcinoma cell lines. Zhongguo yi xue ke xue Yuan xue bao Acta Academiae Medicinae Sinicae. 2004;26(5):519-23.
34. Zargan J, Sajad M, Umar S, Naime M, Ali S, Khan HA. Scorpion (Odontobuthus doriae) venom induces apoptosis and inhibits DNA synthesis in human neuroblastoma cells. Molecular and cellular biochemistry. 2011;348(1):173-81.
35. Deichman GI, editor Early phenotypic changes of in vitro transformed cells during in vivo progression: possible role of the host innate immunity. Seminars in cancer biology; 2002: Elsevier.
36. Abdel-Rahman MA, Omran MAA, Abdel-Nabi IM, Nassier OA, Schemerhorn BJ. Neurotoxic and cytotoxic effects of venom from different populations of the Egyptian Scorpio maurus palmatus. Toxicon. 2010;55(2-3):298-306.
37. Franco R, Cidlowski J. Apoptosis and glutathione: beyond an antioxidant. Cell Death & Differentiation. 2009;16(10):1303-14.
38. Circu ML, Aw TY. Glutathione and modulation of cell apoptosis. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2012;1823(10):1767-77.
39. Akef H, Kotb N, Abo-Elmatty D, Salem S. Anti-proliferative effects of androctonus amoreuxi scorpion and cerastes cerastes snake venoms on human prostate cancer cells. Journal of cancer prevention. 2017;22(1):40.
40. Sifi A, Adi-Bessalem S, Laraba-Djebari F. Role of angiotensin II and angiotensin type-1 receptor in scorpion venom-induced cardiac and aortic tissue inflammation. Experimental and molecular pathology. 2017;102(1):32-40.

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